**PCT** 

REC'D 18 AUG 2004 **WIPO** PCT

# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PU0242-PCT	FOR FURTHER ACTION See	CTION See Form PCT/IPEA/416					
International application No.	International filing date (day/month/ye	ear) Priority date (day/month/year)					
PCT/SE 2003/001127	26.06.2003	28.06.2002					
International Patent Classification (IPC) or national classification and IPC							
C12N 15/10, C07H 1/06							
C12N 13/10, C0/11 1/00	, 60/11 1/00						
Applicant							
Amersham Biosciences	AB et al						
This report is the international pre Authority under Article 35 and tr	eliminary examination report, establishe ansmitted to the applicant according to	d by this International Preliminary Examining Article 36.					
2. This REPORT consists of a total							
	<u> 0</u>						
3. This report is also accompanied b	y ANNEXES, comprising.						
	and to the International Bureau) a tota						
sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).							
sheets which	supersede earlier sheets, but which this	Authority considers contain an amendment that goes					
beyond the d Supplementa	isclosure in the international application	as filed, as indicated in item 4 of Box No. I and the					
b. (sent to the Internation	onal Bureau only) a total of (indicate ty	pe and number of electronic carrier(s))					
_ `	, containing a sequence	listing and/or tables related thereto, in computer					
readable form only, a Administrative Instru		elating to Sequence Listing (see Section 802 of the					
4. This report contains indications r  Box No. I  Basis of	elating to the following items:  of the report						
	•						
Box No. II Priority		the description of the section of th					
Box No. III Non-es	stablishment of opinion with regard to n	ovelty, inventive step and industrial applicability					
	f unity of invention						
applica	Box No. V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement						
Box No. VI Certain	n documents cited						
Box No. VII Certain	n defects in the international application						
Box No. VIII Certain	n observations on the international appli	cation					
Date of submission of the demand	Date of con	npletion of this report					
12.01.2004	03.08.	03.08.2004					
Name and mailing address of the IPEA/S		Authorized officer					
Patent- och registreringsverket	,,,						
BOX 5055 S-102 42 STOCKHOLM Sara Nilsson/Els							
Facsimile No. +46 8 667 72 88		No. +46 8 782 25 00					
Form PCT/IPEA/409 (cover sheet) (January)	Form PCT/IPEA/409 (cover sheet) (January 2004)						



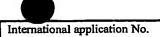


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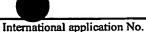
Box	No. I	Ва	sis of the report			
1.	With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.					
		This report is based on a translation from the original language into the following language which is the language of a translation furnished for the purposes of:				
			international search (under Rules 12.3 and 23.1(b))			
		Ħ	publication of the international application (under Rule 12.4)			
		Ħ	international preliminary examination (under Rules 55.2 and/or 55.3)			
2.	furnish	h regard to the elements of the international application, this report is based on (replacement sheets which have been ished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" are not annexed to this report):				
	$\boxtimes$	the inte	ernational application as originally filed/furnished			
		the des	scription:			
		pages		as originally filed/furnished		
		pages*				
		pages*	received by this Authority on			
		the cla	ims:			
		pages	1.1 (Appeth or with a	as originally filed/furnished ny statement) under Article 19		
		pages*		•		
		pages* pages*				
			awings:			
	Ш		awings.	as originally filed/furnished		
		pages*	received by this Authority on			
		pages*				
		a sequ	nence listing and/or any related table(s) - see Supplemental Box Relating to Sequence	e Listing.		
3.		The ar	mendments have resulted in the cancellation of:			
		П	the description, pages			
		Ħ	the claims, Nos.			
		Ħ	the drawings, sheets/figs			
		Ħ	the sequence listing (specify):	<del></del>		
			any table(s) related to the sequence listing (specify):			
4.		This r made, 70.2(c	report has been established as if (some of) the amendments annexed to this report, since they have been considered to go beyond the disclosure as filed, as indicated as).	t and listed below had not been in the Supplemental Box (Rule		
			the description, pages			
			the claims, Nos.			
		同	the drawings, sheets/figs			
		П	the sequence listing (specify):			
		Ħ	any table(s) related to the sequence listing (specify):			
*	If iten	n 4 appli	lies, some or all of those sheets may be marked "superseded."			





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Box No. I	II Non-establishment of opinion with regard to novelty, inventive step and industrial applicability				
The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:					
	the entire international application				
	claims Nos.				
because	e:				
	the said international application, or the said claims Nos.  relate to the following subject matter which does not require an international preliminary examination (specify):				
	_				
	o to the state of				
	the description, claims or drawings (indicate particular elements below) or said claims Nosare so unclear that no meaningful opinion could be formed (specify ):				
	the claims or said claims Nos are so inadequately supported				
	the claims, or said claims Nos are so inadequately supported by the description that no meaningful opinion could be formed.				
$\boxtimes$	no international search report has been established for said claims Nos. 1-8, 10-17 all partially				
	the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:				
	the written form has not been furnished				
	does not comply with the standard				
	the computer readable form has not been furnished				
	does not comply with the standard				
0	the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-bis of the Administrative Instructions.				
	See Supplemental Box for further details.				



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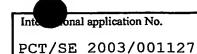
Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.	Statement			•
	Novelty (N)	Claims Claims	1-17	YES NO
	Inventive step (IS)	Claims Claims	_16 _1-15, 17	YES NO
	Industrial applicability (IA)	Claims Claims	1-17	YES NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:

- D1) US4055469
- D2) EP1031626 A1
- D3) Izumrudov V.A. et al, "Controllable stability of DNA-containing polyelectrolyte complexes in water-salt solutions", Biopolymers (nucleic acid sciences), vol. 52, 94-108 (1999)
- D4) Kabanov A. V. et al, "DNA interpolyelectrolyte complexes as a tool for efficient cell transformation, Biopolymers, vol. 31, 1437-1443 (1991)
- D5) Zelikin A. N. And Izumrudov V. A. "Polyelectrolyte complexes formed by calf thymus DNA and aliphatic ionenes: unexpected change in stability upon variation of chain length of ionenes of different charge density", Macromol. Biosc. 2002, 2, 78-81
- D6) EP0281390 A2
- D7) US2002010145 A1
- D8) Ramsden D. K. et al, "Flocculation of cellular material in complex fermentation medium with the flocculant poly(diallyldimethylammonium chloride)", Biotechnology techniques, vol. 12, no. 8, 1998
- D9) US 5010183 A
- D10) BIOSIS, accession number PREV19939610753 "Efficient separation of natural riobonucleotides by low-pressure anion-exchange chromatography"
- D1 shows a method for precipitation of nucleic acids. The method can be used to selectively precipitate nucleic acids from a solution containing proteins. Cationic polymers, e.g. polymers containing quaternary amines are used in the method disclosed. The binding of the polymers to the nucleic acid,



Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of: Box  $\,V\,$ 

the effectiveness of complex formation and precipitation, is not strongly influenced by the pH. To determine the quantity of polymer to be added the quantity of nucleic acids present in the extract can be determined. The effects of charge polymer size on the precipitation and The effect of solutions with different salt investigated. concentration is investigated. Nucleic acids are precipitated leaving other species in the solution. The precipitation is performed on cell lysates. See especially col. 4 lines 39-51, col. 5 lines 36-41, col. 7 lines 30-34, col. 8 lines 13-15 and col. 9 lines 24-32.

D2 shows the isolation of RNA and/or genomic DNA using cationic ammonium salts containing 1-24 repeating units. Nucleic acids are isolated from HeLa cells. The nucleic acid can be separated from the precipitation complex and isolated. See abstract, p. 4 line 18-p. 5 line 26 and p.38 claim 28.

poly(N',N'and e.g. binding between DNA D3the In dimethyldiallylammonium) chloride, ionene bromide or poly(N-The stability of the alkyl-4-vinylpyridinium) is studied. complexes at different salt concentrations is studied. By using a fluorescence spectroscopic assay, the formation of polyelectrolyte complex (PEC) is monitored and the charge ratio when the PEC is formed can be determined (the decrease in fluorescence seen when the charge ratio is about 1 or above A method the salt concentration). depending on the destruction of DNA-containing PECs in watermonitoring salt solutions is also disclosed. It is stated that PECs formed by polycations with quaternary amine groups are pH independent and the least tolerant to destruction of added salt. A mentioned application is delivery of DNA to cells. See p. 97 right col. Paragraph 3, p. 98 right col. and figure 1, p. 99 figure 3, p. 103 and p.105.

D4 relates to methods for increasing DNA hydrophobicity via inclusion into an interpolyelectrolyte complex with polycations. E.g. poly(N-ethyl-4-vinylpyridinium)bromide is used. Conditions under which self-assembly of DNA and polycation occurs, formation of an interpolyelectrolyte

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# Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of:  $Box\ V$ 

complex (IPC), are established. It is shown that the formation of a soluble IPC occurs at a molar ratio of polycation repeating units and nucleic acid groups between 0 and 0,5. Parallel with the soluble IPC an insoluble complex with higher

polycation content is formed. A plasmid is incorporated into an IPC and transformed into cells. See p. 1439 left column.

D5 relates to polyelectrolyte complexes between DNA and aliphatic ionenes. It is stated that the degree of polymerisation and charge density of the ionenes control the stability of the complexes, which might be crucial for applications such as bioseparation. See p. 81 right col. paragraph 2.

D6 shows the use of polycationic solid supports in the purification of nucleic acids form solutions containing contaminants. The cations can be quaternary amines. The bound nucleic acids can be recovered from the support. See p. 6 lines 52-61, p. 7 line 85- p. 8 line 7, p. 17 example 15.

D7 shows a method for selective precipitation of DNA or plasmid DNA by the addition of a compaction agent such as spermidine or spermine. It is stated that the method can be performed on cell lysates. See abstract and fig. 1.

D8 shows the use of poly(diallyldimethylammonium chloride) for flocculation of cellular material. The charge density of the polymer used is 100.

D9 shows a method for purifying DNA or RNA from a mixture of biological materials, which comprises adding a cationic detergent to a mixture. The biological material mixture may be intact cells or cell lysates. The cationic detergent can be a such cationic detergent quaternary amine alkylbenzyldimethylammonium salt. The detergent is added in an dissolve cells, solubilize sufficient to contaminating proteins and lipids in the mixture, and form insoluble hydrophobic complex between the nucleic acid and the detergent. The complex which comprises the RNA or DNA with the detergent is separated from the solubilized contaminants, and may be dissolved or dispersed in a polar organic solvent. Thereafter the DNA or RNA is recovered by the addition of a



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# Supplemental Box

In case the space in any of the preceding boxes is not sufficient. Continuation of:  $Box\ V$ 

salt, which promotes the dissociation of the complex. See col. 2 lines 48-52 and col. 3 lines 24-36.

D10 shows the use of anion exchangers containing quaternary ammonium functionalities for separating riobonucleotides.

The present application relates to the problem of selectively precipitating a nucleic acid from a solution containing other species while leaving said other species in the solution. This is achieved by using a polycationic precipitating agent being a highly charged linear polymer that comprises quaternary amino groups. The method allows precipitation within a broad window of pH values and salt concentrations and is not sensitive to addition of an excess of precipitating agent.

Document D1 is considered to represent the closest prior art.

The difference between the invention according to claim 1 and D1 is that the amount of precipitation agent (such an amount [+]/[-] polycationic between charge ratio about agent and nucleic acid is ≥ precipitating preferably ≥ about 1) used in claim 1 is not is not specified in D1. In D1, the amount of polymer to be added is not determined on the basis of the charge ratio.

The expression "about 1" and "about 0,5" used in claim 1 makes the scope of the claim unclear (see. PCT Art. 6). It is not clear what "about 1" or "about 0,5" means. The optimal charge ratio for forming a specific complex depends upon the salt concentration, but the vague expressions "about 1" and "about 0,5" nevertheless make the scope of the claim unclear.

By adding precipitating agent in the amounts mentioned above, it seems that an insoluble precipitation complex is attained. The precipitation complex is attained within a broad window of pH values and salt concentrations and it is not sensitive to addition of an excess of precipitating agent.

Consequently, with the background of D1, the problem is to attain an insoluble precipitation complex and thus an efficient precipitation in relation to the aspects mentioned



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### Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

above, when performing a precipitation of a nucleic acid using a highly charged linear polymer.

The skilled person faced with the problem mentioned above, finds the solution in D3, which discloses the theory of the stability of polyelectrolyte complexes depending on the charge ratio and the amount of polymer added. D3 shows that the

polymers poly(N',N'-dimethyldiallylammonium) chloride, ionene bromide and poly(N-alkyl-4-vinylpyridinium) bind to DNA and that the stability of the complexes can be controlled by varying e.g. the salt concentration. The skilled person would consider D3 since the document relate to the binding of DNA to polycations, as do D1. The technical application in D3 differs from the application in D1-D2 but the skilled person would combine the documents since they share the same skilled person the obvious to Ιt is bioseparation. in polyelectrolyte complexes can be used Consequently, the invention according to claims 1-3, 5-12 and 17 is considered not to involve an inventive step given what is known form D1 in combination with D3. The addition of salt to dissolve or destruct the complex is investigated in D3. Consequently, the invention according to claims 11-15 considered not to involve an inventive step given what is known from D1 in combination with D3.

The same argumentation as made above can be made starting with D2 or D8-D9 as the document representing the closest prior art. It can be mentioned that D2 and D9 show the recovery of the nucleic acids after separating the precipitate.

In present claim 1 the expression "which method comprises to selectively precipitate the desired nucleic acid, while leaving other species in solution" is used. By using this expression, the method is defined by reference to a result to be achieved by the method, not by technical features characterising how the method is performed. This way of defining the method leads to a lack of clarity (see PCT Art 6).

D1-D2 and D8-D9 show the precipitation of nucleic acids without the use of a strong base. Therefore, it is considered obvious to the skilled person that these methods can be used





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## Supplemental Box

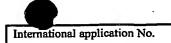
In case the space in any of the preceding boxes is not sufficient. Continuation of: Box  $\,V\,$ 

to precipitate different kinds of nucleic acids. Consequently, the invention according to claim 4 is considered not to involve an inventive step.

Nothing is mentioned in either documents D1-D1 or D8-D9 about isolating more than one desired nucleic acid by continued addition of precipitating agent. Therefore, the invention according to claim 16 is not considered obvious to the skilled person in view of the sited documents.

Documents D4-D7 and D10 are considered to represent the general state of the art.





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# Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The expression "about 1" and "about 0,5" used in claim 1 makes the scope of the claim unclear (see. PCT Art. 6). It is not clear what "about 1" or "about 0,5" means. The optimal charge ratio for forming a specific complex depends upon the salt concentration, but the vague expressions "about 1" and "about 0,5" nevertheless make the scope of the claim unclear.

In present claim 1 the expression "which method comprises to selectively precipitate the desired nucleic acid, while leaving other species in solution" is used. By using this expression, the method is defined by reference to a result to be achieved by the method, not by technical features characterising how the method is performed. This way of defining the method leads to a lack of clarity (see PCT Art 6).